Vol. 12 August 1936 No. 8

Bulletin of the Agricultural Chemical Society of Japan.

TRANSACTIONS

Chemical Studies on the Tannin Substance of Formosan Tea-Leaves.

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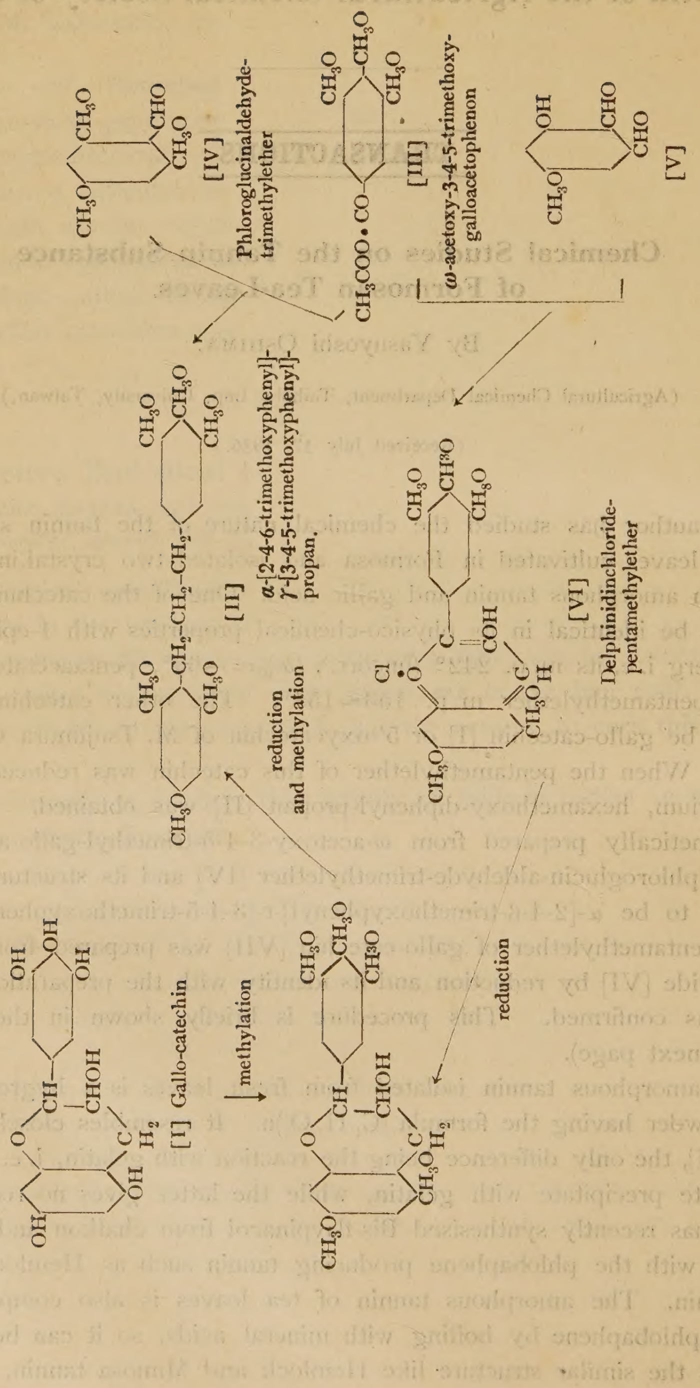
(Received July 17, 1936.)

The author has studied the chemical nature of the tannin substance of fresh tea leaves cultivated in Formosa and isolated two crystalline catechins besides an amorphous tannin and gallic acid. One of the catechins has been proved to be identical in its physico-chemical properties with 1-epicatechin of Freudenberg i. e. its m. p. 242° (uncorr.), $[\alpha]_D = -69°$, pentaacetate m. p. 151 ~ 152°, pentamethylether m. p. 153 ~ 154°. The other catechin has been shown to be gallo-catechin [I] or 5′-oxycatechin of M. Tsujimura $C_{15}H_{14}O_7$ m. p. 227°. When the pentamethylether of this catechin was reduced with metallic natrium, hexamethoxy-diphenyl-propan [II] was obtained. The latter was synthetically prepared from ω-acetoxy-3-4-5-trimethyl-gallo-acetophenon [III] and phloroglucin-aldehyde-trimethylether [IV] and its structure has been confirmed to be α-[2-4-6-trimethoxyphenyl]-r-[3-4-5-trimethoxyphenyl]-propan. Further pentamethylether of gallo-catechin [VII] was prepared from delphinidine chloride [VI] by reduction and its identity with the preparation from tea leaves was confirmed. This procedure is briefly shown in the following scheme: (next page).

The amorphous tannin isolated from fresh leaves is a hygroscopic yellowish powder having the formula $(C_{15}H_{12}O_7)n$. It resembles closely to gallo-catechin [I], the only difference being the reaction with gelatin, i. e. the former gives white precipitate with gelatin, while the latter gives no reaction. A. Russel[1] has recently synthesised Bis-flavpinacol from chalkon and proved its similarity with the phlobaphene producing tannin such as Hemlock and Mimosa tannin. The amorphous tannin of tea leaves is also completely converted to phlobaphene by boiling with mineral acids, so it can be presumed that it has the similar structure like Hemlock and Mimosa tannin. For such



[I] Gallo-catechin

methylation

reduction and methylation

[II] α-[2-4-6-trimethoxyphenyl]-γ-[3-4-5-trimethoxyphenyl]-propan,

[IV] Phloroglucinaldehyde-trimethylether

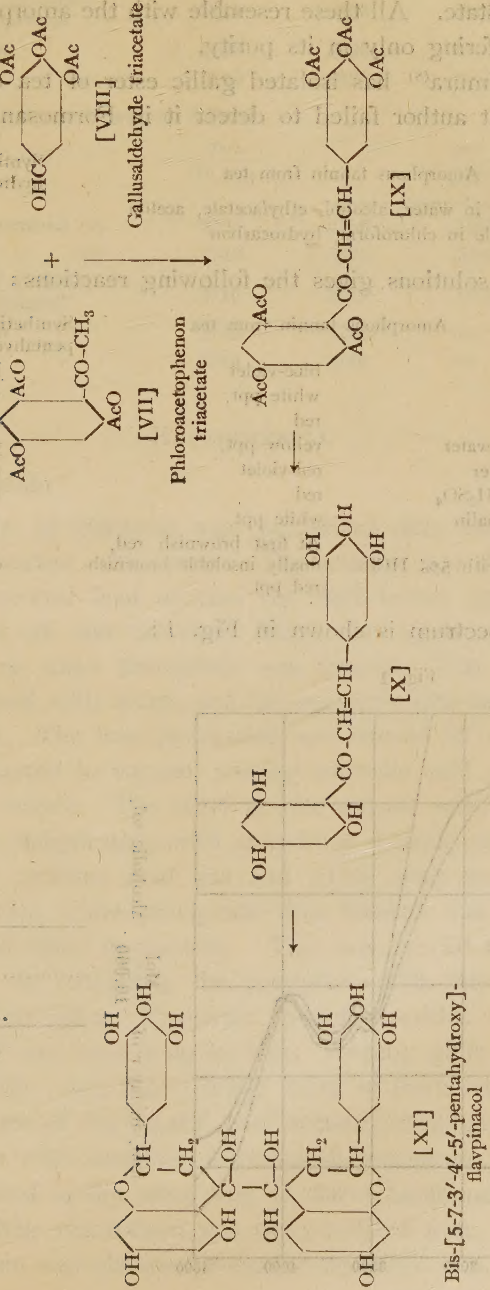
[III] ω-acetoxy-3-4-5-trimethoxy-galloacetophenon

[V]

[VI] Delphinidinchloride-pentamethylether

reduction

a reason the present author has synthesised Bis-[5-7-3′-4′-5′-pentahydroxy]-flavpinacol [XI] according to the method of Russel, by reducing the alcoholic solution of chalkon with zinc and glacial acetic acid and really proved its


[VII] Phloroacetophenon triacetate

[VIII] Gallusaldehyde triacetate

[IX]

[X]

[XI] Bis-[5-7-3′-4′-5′-pentahydroxy]-flavpinacol identity with the amorphous tea tannin. The synthetic procedure is shown in the following scheme (foregoing page):

On tannins of tea leaves, there are many reports of Nanninga(2), Straus(3), Geschwendner(4), Deuss(5), Shaw(6) and Tsujimura(7) has also isolated the tannin in amorphous state. All these resemble with the amorphous tannin isolated by the author, differing only in its purity.

Besides, Tsujimura(8) has isolated gallic ester of tea catechin from green tea but the present author failed to detect it in Formosan tea.

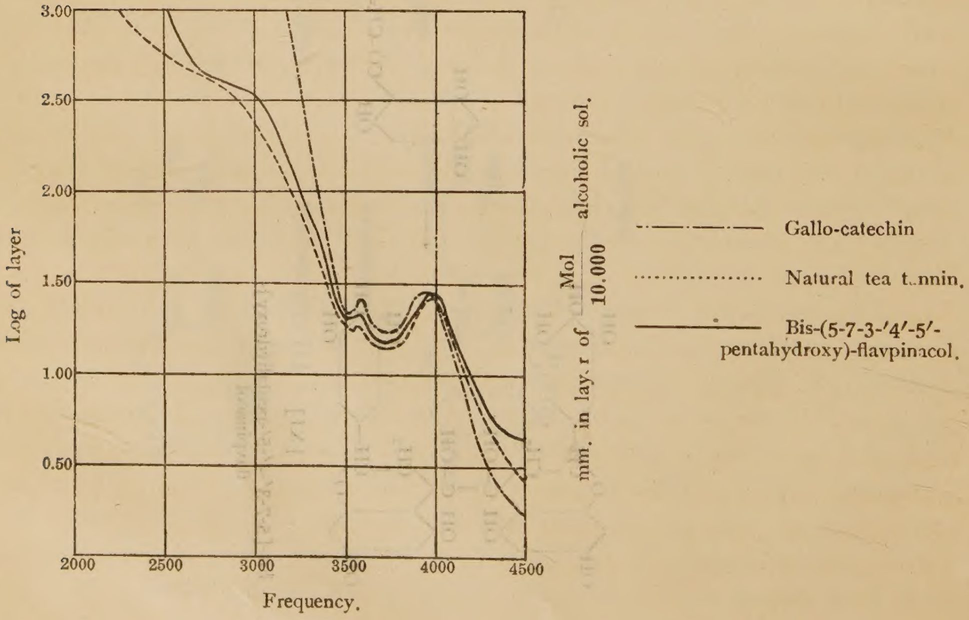
Amorphous tannin from tea	Synthetic Bis-(5-7-3′-4′-5′-hydroxy)flavpinacol
Soluble in water, alcohol, ethylacetate, aceton	do
Insoluble in chloroform, hydrocarbon	do

The aqueous solutions gives the following reactions:

	Amorphous tannin from tea	Synthetic Bis-(5-7-3′-4′-5′-pentahydroxy)-flavpinacol
$FeCl_3$,	blue-violet	blue-violet
Gelatin	white ppt.	white ppt.
KCN	red	red
Bromine water	yellow ppt.	yellow ppt.
Lime water	red-violet	red-violet
Vanillin H_2SO_4	red	red
HCl formalin	white ppt.	white ppt.
Boiling with 5% HCl	At first brownish red, finally insoluble brownish red ppt.	do

Absorption spectrum is shown in Fig. 1:

Fig. 1



Absorption maximum in $\frac{\text{mol}}{10{,}000}$ alcoholic solution

Tea tannin prepared (a)	2700	2790	3480 (inflexion)
〃 〃 (b)	2700	2800	3660 (〃)
Bis-(5-7-3′-4′-5′-penta-hydroxy)-flavpinacol	2700	2800	3500 (〃)
Gallo-catechin	2700	2800	no
l-epicatechin	2700	2820	no

Absorption maximum in aqueous solution

	$\frac{\text{mol}}{10.000}$	$\frac{\text{mol}}{1000}$	
Tea tannin prepared (a)	2720	2720	3480 (inflexion)
〃 〃 (b)	2710	2710	3660 (〃)
Bis-(5-7-3′-4′-5′-penta-hydroxy)-flavpinacol	2710	2710	3550 (〃)
Gallo-catechin	2700	2700	no
l-epicatechin	2780	2780	no

EXPERIMENTAL

Isolation of tea tannin

Fresh tea leaves of Formosa were pulverized after steaming and drying and repeatedly extracted with water. The extract was first treated with a small quantity of neutral lead acetate, the dark brown precipitate produced thereby was filtered off, and the clear filtrate was again treated with neutral lead acetate until no more precipitate was formed. The yellow precipitate was collected, washed with water, and decomposed with hydrogensulphide or with sulphuric acid. The lead precipitate was filtered off and the orange-red filtrate was concentrated in vacuum passing carbonic acid gas and repeatedly shaken with ethyl acetate. The ethyl acetate extract was washed with a little water and, after dehydrating with anhydrous sodium sulphate, evaporated in vacuum passing carbonic acid gas and added with an excess of chloroform. The yellowish white precipitate thus formed was collected, washed with chloroform and dried in vacuum. This was proved to be a mixture of tannin substances. Py extracting the precipitate with ether, gallic acid was obtained in crystalline state. The ether insoluble residue was then dissolved in water, evaporated and kept in an ice box; whereby gallo-catechin separated out in needle crystals : m.p. 227°; yield 0.1% of fresh leaves.

When the filtrate of the neutral lead acetate precipitate was treated with basic lead acetate or with ammonia, a yellowish precipitate was formed. This precipitate was treated in the same way as above mentioned and the precipitate formed by adding chloroform was recrystallized from dilute alcohol. In this way *l*-epicatechin was obtained in needle crystals ; m.p. 242 ; yield 0.003 % of fresh leaves.

Properties

The following reactions were obtained with the aqueous solutions of:

	l-epicatechin	Gallo-catechin	Gallic acid
$FeCl_3$	green	blue violet	blue
Gelatin	no precipitate	no ppt.	no ppt.
KCN	no coloration	orange red	pink
Lime water	purple	purple	greenish blue
Bromine	no coloration	no coloration	no coloration
Vanillin H_2SO_4	red	red	no coloration
HCl Formalin	white ppt.	white ppt.	white ppt.
Reducing power	positive	positive	positive
Soluble in	alcohol, aceton, ethyl acetate.	water, alcohol, aceton, ethyl acetate,	water, ether, chloroform alcohol, aceton, ethyl acetate,
Insoluble in	water, chloroform, ether,	chloroform, ether	

l-epicatechin

The acetyl and methyl derivatives of *l*-epicatechin were prepared after Freudenberg's method.⁽⁹⁾

	l-epicatechin	
	from tea-leaves	after Freudenberg
m p.	242° (uncorr.)	245
$[\alpha]_D$	−69	−68
Pentaacetate m. p.	151∼152°	151∼152°
Pentamethylether m. p.	153∼154°	153∼154°

The absorption spectrum is shown in Fig. 2.

Gallo-catechin

Analysis:

Sample (mg)	H_2O (mg)	CO_2 (mg)	H (%)	C (%)	Camphor (mg)		M
4.885	1.918	10.319	4.39	57.62			
7.038	2.848	14.850	4.52	57.55			
2.149					63.688	4.11°	328.4
0.935					47.111	2.01°	301.7
Calc. for $C_{15}H_{14}O_7$			4.61	58.81			306.1

Specific rotation:

0.7724 g dissolved in 100 cc water $d=1$ dm $t=10°$ $\alpha=-0.29$ $[\alpha]_D -37.5$

0.7356 g dissolved in 100 cc alcohol 1 10 −0.67 −67.0

Acetyl derivative of gallo-catechin

0.2 g gallo catechin were dissolved in 5 cc pyridin, 5 cc acetylchloride was added drop by drop by cooling. The reaction mixture was then poured into water and the white precipitate formed thereby was recrystallized from aceton. It is soluble in aceton, alcohol but insoluble in water; m.p. 189.

Analysis:

Sample (mg)	H_2O (mg)	CO_2 (mg)	H (%)	C (%)
4.982	2.182	10.575	4.90	57.89
4.269	1.808	9.135	4.73	58.36
Calc. for $C_{15}H_9O(CH_3COO)_5$			4.96	58.12

Specific rotation:

0.47 g in 100 cc of aceton at 10 $d=1$ $a=0.10$ $[a]_D=-21.3$

Methyl derivative of gallo-catechin

3 g of the acetate were dissolved in 20 cc methanol and 15 cc dimethylsulphate and 15 cc 50% KOH solution were alternately added drop by drop with stirring passing the current of nitrogen gas. The solution was poured in water and the precipitate formed was recrystallized from aceton; m.p. 184. It is soluble in aceton but difficultly in alcohol.

Analysis:

Sample (mg)	H_2O (mg)	CO_2 (mg)	H (%)	C (%)	AgI (mg)	CH_3O (%)
4.191	2.452	9.803	6.55	62.79		
3.181	1.750	7.331	6.15	62.83		
3.138					9.665	40.70
3.195					9.889	40.90
Calc. for $C_{15}H_9O_2(CH_3O)_5$			5.89	62.75		41.24

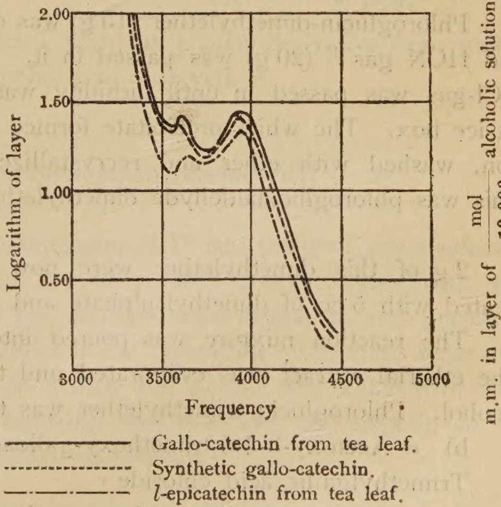
Specific rotation:

0.428 g in 100 cc of aceton, observed at 25°C in 1 dm length $[a]_D=-15.2$

Absorption spectrum

The absorption spectrum was observed with mol/5000 alcoholic solution using Adam Hilger's quartz spectroscope. The maximum absorptions were at 268 $\mu\mu$ and 282 $\mu\mu$ and was hypochromic at 268 $\mu\mu$ to *l*-epicatechin Fig. 2.

Fig. 2.



Reduction of gallo-catechin

Gallo-catechin pentamethylether gives on oxidation a small amount of trimethoxylgallic acid and by kali fusion a trace of phloroglucin. Reducing with metallic natrium, a diphenyl-propan derivative is formed in good yield.

1 g of gallo-catechin was dissolved in 120 cc absolute alcohol, 8 g of metallic natrium were added in small pieces, and heated on a water bath

until the latter completely dissolved. After cooling, the mixture was poured into cold water, acidified with hydrochloric acid and extracted with ether. The etherial extract was eraporated, dissolved in methanol and methylated with 5 cc dimethylsulphate and 5 cc 50% KOH solution. The reaction mixture was poured into cold water and the white precipitate formed thereby was recrystallized from alcohol; m.p. 91; yield 0.6 g. It dissolves easily in ether and hot alcohol but difficultly in cold alcohol.

Kostanecki and Lamp(10) prepared a diphenyl-propan derivative (m p. 87 ~88) from *d*-catechin, but the melting point and analytical result differ from the product obtained by the present authors, as described above.

Analysis:

Sample (mg)	H_2O (mg)	CO_2 (mg)	H (%)	C (%)	AgI (mg)	CH_3O (%)
3.240	2.138	8.053	7.38	67.69		
2.836	1.795	6.991	7.08	67.23		
3.460					11.130	50.14
3.012					11.148	49.91
Calc. for $C_{21}H_{23}O_6$ Hexamethoxy-diphenyl-propan			7.48	67.02		49.97

Comparing with the result of Kostanecki and Lamp the structure of the reduction product of gallo-catechin is assumed to be *α*-[2-4-6-trimethoxy-phenyl]-*γ*-[3-4-5-trimethoxy-phenyl]-propan. This assumption was further confirmed by synthesis.

Synthesis of the diphenyl-propan derivative

a) Phloroglucinaldehyde-trimethylether [IV]

Phloroglucin-dimethylether (15 g) was dissolved in 200 cc absolute alcohol and HCN gas(11) (20 g) was passed in it. After boiling for some times, dry HCl-gas was passed in until turbidity was produced and left over night in an ice box. The white precipitate formed thereby was collected by decantation, washed with ether and recrystallized from water and dilute alcohol. This was phloroglucinaldehyde dimethylether [V](12)(13)(14); m. p. 70°; yield 10 g.

2 g of this dimethylether were now dissolved in 10 cc methanol and treated with 5 cc of dimethylsulphate and 5 cc of 50% KOH solution.

The reaction mixture was poured into water and extracted with ether. The etherial extract was evaporated and the residue was recrystallized from alcohol. Phloroglucin trimethylether was thus obtained: m.p. 75°; yield 2 g.

b) *ω*-Acetoxy-3-4-5-trimethoxy-galloacetophenon III

Trimethylgallic acid chloride

10 g of trimethylgallic acid were mixed with 10 g of thionylchloride and kept for 24 hours at room temperature. The excess of thionylchloride was expelled off under reduced pressure at 50°, the residue was subjected to

vacuum distillation. The fraction boiling at 177° by 10 mm was trimethylgallic acid chloride: m p. 77～78°(13); yield 10 g.

w-Diazo-3-4-5-trimethoxy-galloacetophenon(13)(6)(17)

6 g of trimethylgallic acid chloride were dissolved in 12 cc of chloroform and treated with 1.2 g of diazomethan. After sanding over night in an ice box, yellowish crystals separated out: m p. 103°; yield 3.2 g.

w-Acetoxy-3-4-5-trimethoxyacetophenon [III](13)(16)(17)

3 g of diazo compound obtained as above were dissolved in 6 cc of glacial acetic acid and heated at 80～90°, whereby the acetoxyl compound is formed evolving nitrogen gas. The solution was now poured into cold water and the precipititate thus produced was recrystallized from alcohol; m.p. 86°; yield 2.5 g.

c) Diphenyl-propan derivative

Phloroglucinaldehyde-trimethylether [IV] and *w*-acetoxy-3-4-5-trimethoxyacetophenon [III] in etherial solution is treated with metallic natrium in molecular proportion(15) and heated on a water bath for 24 hours.

Then the excess of ether was decanted and the yellow precipitate was dissolved in glacial acetic acid and reduced with platinum black(18).

The residue of the solution was crystallized from alcohol. It was white plate crystals; m.p. 91°, soluble in ether, alcohol and insoluble in water; yield 0.3 g from 1 g of acetophenon.

Analysis:

Sample (mg)	H_2O (mg)	CO_2 (mg)	H (%)	C (%)
3.212	2.160	7.988	7.53	67.02
Calc. for $C_{21}H_{28}O_6$			7.45	67.02

By mixing the synthetic specimen with the propan derivative from gallocatechin no depression of melting point was observed.

Synthesis of gallo-catechin

I 3-oxy-5-7-3′-4′-5′-pentamethoxyflavyliumchloride [VI]
(delphinidin-pentamethylether)(6)(7).

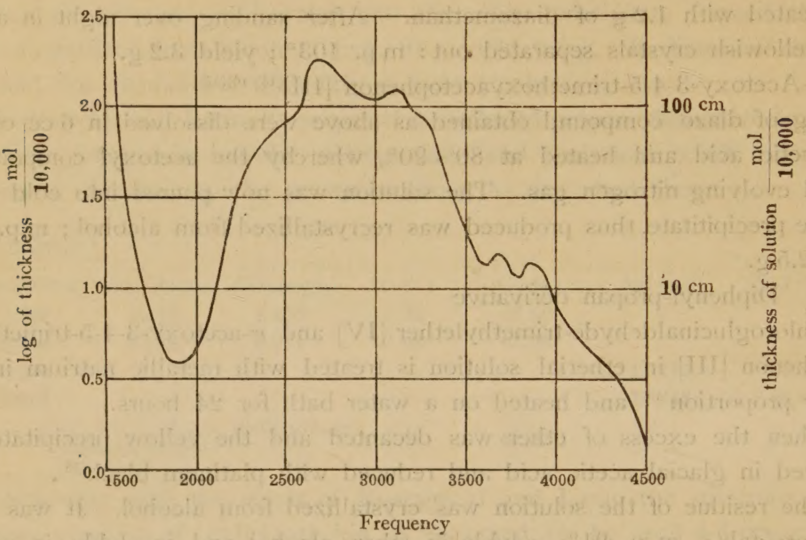
1 g of *w*-acetoxy-trimethoxyacetophenon [III] and 0.6 g of phloroglucindimethylether [V] were dissolved in dry ethyl acetate and treated with dry HCl gas. After two hours when the solution was turned to red purple it was kept in an ice box whereby a violet black crystalline precipitate separated out, yield 0.6 g. It was dissolved in a little hot alcohol and added with concentrated HCl drop by drop until turbidity was produced and kept in an ice box. The crystals separated out melted at 204～6.

Analysis:.

Sample (mg)	H_2O (mg)	CO_2 (mg)	H (%)	C (%)
2.801	1.021	5.376	4.08	52.35
Calc. for $C_{20}H_{21}O_7Cl$. 21/2 H_2O			4.63	52.92

Absorption spectrum of this flavyliumchloride was quite identical with delphinidin-pentamethylether as shown in Fig. 3.

Fig. 3.



II 5′-oxy-catechin

0.5 g of the flavyliumchloride above obtained (I) was dissolved in glacial acetic acid and reduced with 0.1 g of platinum black. After evaporation the residue was dissolved in warm alcohol, added a little water and kept in an ice box. White needle crystals separated out was recrystallized from aceton ; yield 0.1 g, m. p. 183, optically inactive.

Analysis :

Sample (mg)	H_2O (mg)	CO_2 (mg)	H (%)	C (%)
2.908	1.503	6.651	5.78	62.38
Calc. for $C_{15}H_9O_2(CH_3O)_5$			5.89	62.75

The synthetic product was proved to be identical with natural gallo-catechin except optical activity, i. e. the specific rotation of the latter being $[\alpha]_D = -15.2$.

Isolation of the amorphous tannin.

a) When the tannin substance prepared in the manner as reported in the previous part was dissolved in water and saturated with sodium chloride, gallic acid crystallized out ; m. p. 238. The filtrate therefrom gave on cooling in an ice box yellowish white crystals which after recrystallization from hot water formed needle crystals of gallo-catechin (m. p. 227). The filtrate was further concentrated and kept in an ice box but no more crystals separated out, so it was repeatedly shaken with ethyl acetate. When the ethyl acetate extract was concentrated, and added with a large quantity of chloroform, yellowish precipitate of tannin was obtained, which was collected, washed with chloroform and ether, and dried in vacuum; very hygroscopic yellowish powder; yield, 0.4% of fresh leaves.

b) Another method of isolation :—

According to the method of Deuss(5) and Shaw(6) fresh intact tea leaves were repeatedly extracted with warm water at 60°. The extract was concentrated in vacuum and after shaking with benzene the aqueous layer was saturated with sodium chloride and repeatedly extracted with ethyl acetate. The ethyl acetate extract was treated in the same way as described above. The amorphous tannin was thus obtained accompanying a little gallo-catechin and gallic acid.

Analysis: Samples were dried in vacuum at 100° for 4 hours

	Samples (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
Prepared by the method (a)	5.813	12.430	2.354	58.32	4.53
〃 〃 (a)	4.209	8.889	1.741	57.60	4.52
〃 〃 (b)	6.512	14.048	2.601	58.83	4.47
Calc. for $C_{15}H_{14}O_7$				58.81	4.61

Synthesis of Bis-(5-7-3′-4′-5′-pentahydroxy)-flavpinacol [XI]

10 g of phloroacetophenontriacctate(19) [VII] and 9 g of gallusaldehydetriacetate 20) [VIII] were dissolved in 80 cc of dry ethyl acetate, cooled and saturated with dry HCl gas. The solution turned thereby from red to orange yellow. The ethyl acetate was now removed by evaporation and the residue was crystallized from dilute alcohol. Chalkon acetate was thus obtained as brown crystals [IX] yield, 10 g; m p. 184.

9 g of chalkon acetate were dissolved in 300 cc alcohol and boiled with 300 cc of 10% KOH solution in the atmosphere of nitrogen gas. Then it was evaporated in vacuum to a half volume, acidified with HCl and extracted with ethyl acetate. The ethyl acetate extract was then washed with water, dehydrateed with natrium sulphate and evaporated. The crystalline residue thus obtained was recrystallized from .dilute alcohol; m p. 278 [X]; yield, 1.5 g.

Analysis:

Sample (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
3.252	7.036	1.174	58.68	4.04
Calc. for $C_{15}H_{12}O_7$			59.21	3.95

lg of chalkon was dissolved in 30 cc of boiling alcohol and treated with 7 cc of glacial acetic acid and 5 g of zinc dust. The solution was then rapidly cooled, diluted with water, saturated with sodium chloride and extracted with ethyl acetate. The brownish orange colored extract was, after washing with a little sodium bicarbonate and water, evaporated in vacuum passing CO_2 gas. The residue was dissolved in water, saturated with sodium chloride and again extracted with ethyle acetate. When the ethyl acetate extract was dried, concentrated and mixed with a large volume of chloroform, a yellewish precipitate was produced, which was collected and dried in vacuum. It was a yellowish, amorphous, very hygroscopic powder.

For analysis, the sample was dried in vacuum at 100° for 4 hours.

Anlysis:

Sample (mg)	CO_2 (mg)	H_2O (mg)	C (%)	H (%)
4.930	10.593	2.188	58.60	4.97
4.618	9.971	2.060	58.89	4.97
Calc. for $C_{30}H_{26}O_{14}$			59.02	4.26

The author expresses his sincere thanks to Prof. R. Yamamoto for the kind advice and encouragement throughout this work.

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ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

Experimentelle Untersuchungen über den Einfluss von Radium-Bestrahlung auf die Gärungsmikroorganismen. (Vorläufige Mitt.)—Die Bildung konstanter Variation der Heferassen unter der Einwirkung von Hokutolit-Strahlen. (S. 699～708): Von Ryôdi NAKAZAWA und Mituo SIMO. (The Department of Industry, Government Reserch Institute, Taiwan, Japan; Received Apr. 13, 1936.)

Als Vorversuch der gärungsmikrobiologischen Untersuchung mit Radiumstrahlen wurde der Einfluss der Hokutolit-Strahlen auf die Hefe untersucht, wobei eine Bildung konstanter Variation der Heferassen beobachtet wurde.

Diese konstanter Heferassen sind morphologisch wie physiologisch deutlich von ursprünglichen Stämmen verschieden. Man bemerkt nämlich eine Entartung in der optimalen Temperatur, Abtötungstemperatur, Sporenbildung und Vergärbarkeit oder Nichtvergärbarkeit der Zuckerarten. Bei der Prüfung des Gärungsvermögens gegenüber Glukose, Saccharose und Maltose zeigte sich, dass das Gärungsvermögen von 5 Arten unter den entarteten Stämmen im Vergleich mit den ursprünglichen Stämmen herabgesetetzt war; dagegen hatte 1 Stamm stets ein grösseres Gärungsvermögen als die ursprünglichen Stämme.

The Influence of Monochromatic Lights on the Action of the Fat-Splitting Enzyme in the Yeast. (pp. 709～713): By Reitaro MURAKAMI. (Agricultural College, Utsunomiya; Received Apr. 17, 1936).

In order to investigate the influence of the spectoral monochromatic lights on the action of the fat-splitting enzyme in the yeat, the author added 5 c.c. of enzyme solution into the test tubes containing 5 c.c. of castor oil, which neutralized with N/20 NaOH solution. Enzyme solution was prepared by the autolysis of the pressed yeast and the maceration of the dried "Oriental yeast".

The tubes were placed with in the tinned boxes, at the front windows of which the monochromatic filters of red, yellow, green, blue, violet and ultra vit glass were placed. The filters of white and black passing respectively all

and no visible rays were also applied as the controls. The boxes containing the test tubes were incubated at 37°C. and lighted by an electric lamp through the layer of copper salt solution and the filters.

The tubes were taken out from each box at the intervals of 12, 24 and 48 hours after the incubation and then the end product, fatty acids of the hydrolysis were titrated using N/20 NaOH solution by Knitz's method.

In this experiment the apparent effect on the fat-splitting enzyme in the yeast was found to be almost stronger under the rays of longer wave length, i. e. the red or yellow regions and it was gradually weaker under the rays of shorter wave length, i. e. green–violet. The action was most inferior in the dark.

But the rates of the fatty acids isolated by the fat-splitting enzyme for an unit intensity of the rays was found to be in inverse effect in comparison with their intensities.

Hence, the rays present the positive stimulant action for fat-splitting enzyme in the yeast, but the effect has been decreased successively with their intensities.

Studies on Soya-been Oil-foots.—(1) Isolation of Stachyose and an organic Complex. [oils-phophatides-fatty acids-ashes-(glucose)]. (pp. 714～720): By Kozi OKANO, Iwao OHARA and Jiro KATO. (The Central Laboratory of the South Manchuria Railway Co.; Received Apr. 30, 1936.)

The authers have investigated on the composition of oil-foots obtained as a by-product of the so-called alcohol extraction process of soya-bean oil, and found that they were composed of on an average 11% of moisture, 16% of soya-bean oil, 45% of Stachyose, and 24% of an organic "complex".

Stachyose was easily separated from the "complex" by means of 60% alcohol and isolated in the crystalline pure state. The "complex" when purified was a colourless amorphous substance in which the following several compounds were loosely bound together to form a complex: phosphatides, fatty acids, fats, inorganic acids and glucose.

The above mentioned phosphatides were separated into three fractions which were analysed for their decomposition products, none of those fractions being identical with lecithin or cephalin. In the oil fraction two kinds of triglycerides, that is liquid and solid, are found and they are distinguished from normal soya-bean oil by the solubilities in absolute alcohol, in which they easily dissolve at ordinary temperature. The inorganic acids were mostly made of phosphoric and silicic acids and the fatty acid probably of myristic acid.

The Chemical constituents of Actinidia Callosa Lindl. var. rufa Makino. I. (pp. 721～725): By Yoshijiro KIHARA. (Agricultural Chemical Laboratory, Tokyo Imperial Universiy; Received Apr. 22, 1936.)

The mucilage of Actinidia Callosa Lindl. var. rufa Makino has been used for paper-making from an ancient time at the Southern part of Japan.

The analysis of the bark was as follows: moisture 8,88% ether extract 0,89%, unsaponifiable matter in the ether extract 0,38%, total soluble carbohydrates 19,89%, pentosan 10,20%, crude fibre 64,56%, ash 6,52%, nitrogen 0,50%, crude protein 3,13% and tannin 1.92%.

The powdered bark was succesesively extracted with hot 95% alcohol, cold water, hot water and 5% KOH solution. The distribution of carbohydrates in each fraction was determined.

			dry matter (%)
1.	Hot alcohol extract	sugars	0,70
		pentose	0
2.	Cold Water extract	sugars	5,72
		pentose	0,75
3.	Hot water extract	sugars	0,57
		pentose	trace
4.	5% KOH solution extract	sugars	1,84
		pentose	0,20
5.	Residue	sugars	10,64
		pentose	12,11

A mucilage was precipitated with alcohol from the cold water extract. It consisted of 4.4 parts of galactose and one part of arabinose. Galactose in the hydrnlysate of the mucilage was proved as phenylosazone, *o*-tolyhydrazone, and by the formation of mucic acid and the isolation of pure galactose crystal. After removal of galactose crystal from the hydrolysate, arabinose was obtained as benzylphenylhydrazone.

The mucilage gave neither Seliwanoff's reaction, naphthoresorcin reaction nor iod reaction. It was precipitated from aqueous solution with $Ba(OH)_2$ or Fehling's solution. No. precipitate was obtained by saturating with $(NH_4)_2SO_4$. It was easily hydrolyzed by the enzyme of the snail exttract, while takaiastase had no action.

A remarkable amount of pentosan was extracted by heating the extraction residue with water at 140°C. The hydrolysis of the pentosan gave arabinose which was idedtified as benzylphenylhydrazone.

A colorless needle macroscopically observed on the bark was identified with Ca oxalate.

Tannin isolated from the bark was proved to be one belonging to pyrocatechin.

Untersuchungen von Eissigsäurebakterien aus Formosa (X).

—Systematische Untersuchungen über Essigsäurebakterien aus Formosa. (1). (S. 726~744): Von Syosuke TANAKA. (Department of Industry, Government Reserch Institute, Taiwan, Japan; Received Apr, 5, 1936.)

Verf. hat 105 Essigsäurebakterien aus verschiedenem Material aller möglichen Gegenden Formosas isoliert und sie morphologisch und physiologisch untersucht.

Bei der Kultur dieser Essigsäurebakterien in einer Nährflüssigkeit, die Weingeist und Traubenzucker enthält, wurde entdekt, dass je nach der Art dieser Bakterien die vier folgenden Oxydationsvorgänge der beiden genannten Komponenten sich unterscheiden lassen, nämlich

(a) Solche Bakterien, die nur flüchtige Säuren, aber keine nichtflüchtigen Säuren bilden,
(b) solche, die die Bildung der flüchtigen Säuren zu Ende führen und erst nach dem Aufhören weiterer Peroxdation dieser flüchtigen Säuren nichtflüchtige Säuren bilden.
(c) solche, die nach Beedigung der Bildung von flüchtigen Säuren die nichtflüchtigen Säuren entstehen lassen,
(d) solche, die gleichzeitig flüchtige und nichtflüchtige Säuren bilden.

Nachdem sich Verf. überzeugt hatte, dass diese Spezifität auf der Individualität der Bakterien beruht hat er sie als Typus der Essigsäurebakterien bezeichnet und sie in Typus I, Typus II, Typus III und IV unterschieden. Bei der Klassifizierung der in Formosa vorkommenden Essigsäurebakterien nach obigen Typen ergab sich, dass Abtötungstemperatur, Alkoholfestigkeit und Essigsäurefestigkeit bei den zum Typus I, II, III gehörigen Bakterien hoch bezw. gross sind und die Menge gebildeter Essigsäure gross ist. Was die Menge entstandener Glukonsäure anbetrifft, so ist dieselbe beim Typus I gleich Null, beim Typus II sehr gering, beim Typus III jedoch etwas grösser, aber in allen Fällen geringer als die gebildete Essigsäuremenge. Die zum Typus IV gehörigen Bakterien sind sehr mannigfach, aber die Abtötungstemperatur, die Alkoholfestigkeit und Essigsäurefestigkeit sind allgemein niedrig und die gebildete Nenge an Glukonsänre ist grösser als die der Essigsäure.

Auf Grund obiger Resultate glaubt Verf., dass mann durch Untersuchung der Typen dieser Essigsäurebakterien eine Klassifizierung, die der physiologischen Wirkung der Essigsäurebakterien am besten entspricht, machen kann.

On the Correlation of the organic and the mineral Matters contained in Malberry Leaves.

(pp. 745~748): By Kiyotoku KATO. (The Agricultural Experimente of Gifu Prefecture; Received Apr. 27, 1936.)

Writer studied on the correlation of the organic and the mineral matters contained in malberry leaves, and found the correlation exist as follows.

Results are as Follows.

1. The quantity of K_2O, P_2O_5 and sulfuric acid are correlated to the quantity of the raw proteine and Na_2O to the soluble carbohydrate in sp. gr. 1.15 HCl. The CaO and SiO_2 are correlated to the quantity of raw proteine negatively.

2. The correlation co-efficient and probable error of correlation coefficient are as follows.

(a) Between raw protein and P_2O_5 $\gamma=0.67$ $E_\gamma=0.09$
(b) Between raw protein and K_2O $\gamma=0.75$ $E_\gamma=0.06$
(c) Soluble carbohydrate and Na_2O $\gamma=0.82$ $E_\gamma=0.04$

Die Untersuchungen über die Mikroorgamismen von Zukerrohrsäfte. (S. 749~762): Von R. NAKAZAWA, Y. TAKEDA unn K. SUEMATU. (The Department of Industry, Government Reserch Institute, Taiwan, Japan. Zugeg. am 22 Apr. 1936.)

(1) In 1 ccm Zuckerrohrsaft existieren 10,000 bis 100,000 Mikroorganismen, die alle Invertase haben und Rohrzucker invertieren.

(2) Was Mikroorganismen anbetrifft, so sind sie Saccharomyces, Torula, Monilia und Bakteria (hauptsächlich Leuconostoc) etc.

(3) Temperatur, Zuckerkonzentration, pH etc. des Zuckerrohrsafts eignen sich sehr gut für die Vermehrung der Mikroorganismen.

(4) Man kann den Wachstum dieser Mikroorganismen durch Aenderung des pH, durch Temperaturerhöhung und durch Chlorkalkzusatz ändern.

Studies on the Amylase of Yeast. XIII—Summary. (pp. 763~772): By Kazuki ONO. (Biochemical Laboratry of Taihoku Imperial University, Japan; Received Apr. 22, 1936.)

On the exchangeable Cation Content as related to climatic Soil types in Manchuria. (pp. 773~782): R. KAWASHIMA. (Agri. Chem. Laboratory, Kyûshû University; Received Apr. 30, 1936.)

The exchangeable Cations in four Soils of different types in Manchuria were determined. The Soil reaction, exchange Capacity in mg. equivalent per 100 g of dry soil and percentage compositions of exchangeable cations are summarized as follows :

(1) **Steppe Soil**, from Sekiho in Jehol.

Horison	pH		Exchange Acidity	Hydroly. Acidity (y′)	Exchange Capacity	Percentage Composition of cations			
	H_2O	KCl				Ca	Mg	K	Na
A_1	8.02	7.35	basic	1.6	30.10	86.3	8.2	1.8	3.6
A_2	7.60	6.96	〃	1.6	35.75	86.8	9.1	1.2	2.8
C	7.74	7.24	〃	1.2	22.57	84.9	10.3	1.8	4.0

(2) **Steppe Soil**, Changed from forest-type soil deforested many hundreds years ago.

Horizon	pH		Exchange Acidity (y′×3)	Hydroly. Acdity (y′)	Exchange Capacity	Percentage Composition of cations				
	H_2O	KCl				H	Ca	Mg	K	Na
A_1	7.90	7.20	basic	1.4	30.89	0	91.5	7.5	0.5	0.5
C	7.11	5.92	0.3	5.0	26.61	11.9	69.5	17.7	0.7	0.2

This soil was collected in Sogakukohu, about 150 km. north-westward of city Tonan. Its C-horizon is slightly acidic and still retains the appearnce of forested age.

(3) **Brown Forest Soil**, from Hokuantin about 300 km. northward of city Harbin.

Horizon	pH		Exchange Acidity (y′×3)	Hydroly. Acidity (y′)	Exchange Capacity	Percentage Composition of cations					
	H_2O	KCl				H	Ca	Mg	Mn	K	Na
A_1	5.76	4.81	1.0	26.5	33.68	9.5	65.4	22.8	0.3	1.0	1.0
A_2	4.96	4.18	3.2	34.1	29.65	23.7	49.2	24.1	0.2	1.2	1.5
B_1	5.06	4.08	8.0	22.8	26.33	25.1	44.6	27.1	0.2	1.3	1.7
B_2	5.12	4.06	3.6	14.7	26.20	18.1	50.3	28.4	0.1	1.4	1.7
C	5.24	4.34	2.3	13.4	24.93	16.0	51.4	29.0	—	1.4	2.1

The A_1 horizon is regenerating owing to the deforestation since very long years ago. The lower horizons react acidic and their relative amounts of calcium are low.

(4) **Podzol**, from Sinko onthe Tonei railway line in south-eastern part of Manchuria.

Horizon	pH		Exchange Acidity	Hydroly. Acidity	Exchange Capacity	Percentage Composition of Cations					
	H_2O	KCl	(y′×3)	(y′)		H	Ca	Mg	Mn	K	Na
A_1	6.76	6.07	0.7	11.0	26.98	1.5	82.3	13.3	0.1	0.3	2.5
A_2	5.56	4.65	0.8	7.1	16.29	24.5	55.9	11.9	——	4.3	3.4
B_1	5.86	4.60	0.6	7.2	21.64	19.7	60.4	16.7	——	1.1	2.1
B_2	5.68	4.13	1.5	9.8	29.17	21.1	57.6	17.9	——	0.8	2.6
B_3	5.77	4.20	2.5	10.9	32.86	22.3	56.8	16.9	——	1.0	3.0

Owing to influence of deforestation the uppermost horizon is losing the podzolic nature and securing steppe-like character. But, in the horizon A_2, the bleached layer, the exchange capacity is the lowest and relative content of hydrogen is the highest. It is one of the proper characters of Podzol.

Enzymatic Studies on Cereals. Part V.—On the Saccharifying Amylase of rice. (pp. 783～792): By Gohei YAMAGISHI. (Morioka Imperial College of Agriculture and Forestry; Received, Apr. 20, 1936).

In the previous paper, the auther has mainly reported the starch liquefying enzyme of rice.

The present experiment has been carried out to study from the standpoint of the saccharifying power on the amylase in the rice grain.

The saccharifying power was determined by measuring the reducing substances formed by the action of the enzyme, by means of Willstätter and Schübel's hypoiodite method.

The results obtained from this investigation may be summarized as follows: —

(1) It could be decided that the sacchrifying Amylase was present in the aqueous extract of the unhulled rice and uncleaned rice flour.

(2) For the extraction of the sacchrifying amylase with water, it was suitable lower temperature and shoter digestion time than that for the extraction of the liquefying amylase; i. e. the extraction for about 10 hour in the ice-chest has brought out the best results.

(3) The optimum hydrogen ion concentration of the saccharifying amylase in the aqueous extract of the unhulled rice, uncleaned rice, aed rice-bran lies in about 5.0, 4.7, and 6.6 pH respectively.

When the aqueous extract is purified by means of dialysis or precipitation with alcohol, the optimum pH is transformed to acidic side; namely the amylase from the unhulled rice, uncleaned rice, and rice-bran is the most active at the pH 4.8, 4.6, and 4.8 respectively.

(4) Under the author's experimental conition (4 hours and 4.8 pH), the

optimum temperature of the saccharifying amylase is 45°C.

(5) The Saccharifying amylase is mose sensitive to heat than the liquefying amylase.

Enzymatic Studies on Cereals. Part VI.—On the Saccharifying Amylase on the well-cleaned Rice. (pp. 793∼799): By Gohei YAMAGISHI.

(Morioka Imperial College of Agriculture and Forestry; Received Apr. 20, 1936).

As described in the 4 th report of this series the liquefying amylase differs in the distribution and form according to its position in the rice-grain.

The object of the work here reported is to ascertion whether or not the distribution and form of the saccharifying amylase in the rice-grain is similar in the case of the liquefying amylase, at the same time effect of various inorganic and organic salts on the extraction of the enzyme from the wellcleaned rice flour.

The results is as follows:

(1) Studies were made on the saccharifying amylase in the uncleaned rice and cleaned rice, polished with different grade, using Willstätter and Schübel's hypoiodite method.

(2) The optimum pH of the saccharrifying amylase in the extract form the wellcleaned rice flour with salt or papain solution, lies both in about 4.6 and the dialysate and the precipitated amylase with alcohol show the maximum activity at the pH 4.5 and 4.3 respectively.

(3) The form of the saccharifying amylase differs according to the exterior or interior of the grain; in the outer part nearly all the enzyme is readily soluble in water but in the inner part it is present in an insoluble from in combination (so-colled amylase-zymogen) which may be liberated by adding papain or salt.

(4) It was found that there was the optimum concentration, extracting the amylase zymogen in the wellcleaned rice flour with the salt or papain solution.

(5) It was confirmed that except in very rare instances all the inorganic and organic salts (0.1 M solution) showed a remarkable effect on the extraction of the saccharifying amylase from the wellcleaned rice flour.

(6) Generally speaking, these results coincide with the fact that has previously been ascertained by the author in the case of the liquefying amylase.

Chemical Studies on Silk-Fibroin (II)—Generality of Fibroin-Components. (pp. 800∼806): By Hideo KANEKO and Tyuitiro KOMATSU. (Imperial College of Sericulture and Silk-Industry, Ueda, Japan; Received Apr. 14, 1936.)

Chemical Studies of the Amino Acid. III.—The apparent Dissociation Constants of Canavanine and Canaline. (pp. 807∼813): By Tetuo TOMIYAMA. (The Imperial Fisheries Institute; Received Apr. 20, 1936.)

1. The apparent dissociation constants and the corresponding free energy changes for canavanine and canaline have been determined at 25°.

2. It is shown that the basic group of canavanine is far weaker than the guanidine group of arginine and a little stronger than the imidazole group of histidine. The basic group of canaline other than the α-amino group is so weak that it is a little difficult to distinguish it from the α-amino group.

3. The isoelectric points (pI) of canavanine and canaline have been determined to be 7.93 and 6.45, respectively.

4. The theoretical interpretation of the data was so far advanced that canaline may be a derivative of hydroxylamine, and that the oxygen bridge of canavanine may play an important rôle in decreasing the bacicity of the guanidine group.

Chemical Studies of the Amino Acid. IV.—On the Nature of the Reaction which takes place between certain Amino Acids and Formaldehyde. (pp. 814∼820): By Tetuo TOMIYAMA. (The Imperial Fisheries Institute; Received Apr. 25, 1936.)

1. The calculation of the equilibrium constant of the reaction which takes place between certain amino acids and formaldehyde shows that 1 molecule of amino acid combines with 1 molecule of formaldehyde from pH 10.0 to 8. The values for glycine, alanine, and proline are 437 ± 18, 50.1 ± 4.9, and 67.3 ± 3.4, respectively.

2. The mechanism of the reaction in question is discussed. The reaction product most probably may be a molecular compound and not a methylene compound.

Chemical Studies of Amino Acid. V.—The Solubilities of *l*-Proline and *l*-Hydroxyproline in Water, the Calculated Heats of Solution, and the Apparent Molal Volume of *l*-Hydroxyproline. (pp. 821～827): By Tetuo TOMIYAMA. (The Imperial Fisheries Institute; Recdived May 8, 1936.)

1. The solubilities at various temperatures of *l*-proline and *l*-hydroxyproline has been determined. A micromethod has been developed in using a special pipette for weighing the sample and measuring its volume.

2. The solubility temperature relationships within the temperature limits in which solubility estimations were carried out can be represented by the following equations.

l-proline $\ln N_2 = -3.8586 + 0.7586 \times 10^{-2} T$
l-Hydroxyproline $\ln N_2 = -5.5906 + 0.8514 \times 10^{-2} T$

3. The differential heat of solution of *l*-proline and of *l*-hydroxyproline has been calculated to be 1340 calories and 1506 calories, respectively.

4. The apparent molal volume of *l*-hydroxyproline in its saturated solution has been calculated from the density data. The mean value of the apparent molal volume was 85.6±0.12. The molal volume calculated from the Traube's empirical atomic volume is 85.2. This agreement most probably shows that the tension and the contraction of the pyrrolidin ring of *l*-hydroxyproline may be negligibly small, in other word, the ring of *l*-hydroxyproline molecule may exist in quite a normal state.

Nutritive Value of Canavanin (amino acid) **(Influence of Canavanin on Pregnancy and Parturition.)** Report VI. (pp. 828～832): Masayoshi OGAWA. (Dept. of Nutrition Tokyo Municipal Hyg. Lab.; Received May 1, 1936.)

In the previous communications the author reported that amino acid called Canavanin was an essential nutritive element for the growth in the early life of the animals, but not essential in the later life of the growing period. In order to study the growth promoting potency of Canavanin, Kitagawa fed young albino rats on a basal diet free from vitamin G and substitue Canavanin instead of that vitamine. But he could not obtained the normal growth of animals. It is a clear fact that how presumably adequate in other respects the animals kept upon the diet free from vitamin G, will inevitably cease to gain in body weight, and no amino acid will be able to restore the animal to the normal growth. Therefore Kitagawa's experiment seems to be entirely different from the author's.

In the present report, the author tried to determined an influence of Canavanin on pregnancy and parturition. He employed a number of male and female well grown albino rats and divided the animals into 2 groups. He kept each pair in the same cage and fed on a diet deficient in Canavanin for a certain period. To the group I, gave Canavanin 0.1 g per kilo gram body weight per day during the experiment, while to the group II, did not give Canavanin at all.

From those two groups of animals the author obtained the following results.

1) There was no difference in the rate of pregnancy between the group I (animals fed on Canavanin) and group II, (animals not fed on Canavanin).

2) The animals which fed on Canavanin were able to deliver of youngs but all of the youngs were died, the animals not fed on Canavanin could able to be pregnant, but every one of them were died before delivere of youngs. The rate of delivered of youngs of the group I was 66% while the group II was zero.

Conclusion

As shown in the above reuslts, Canavanin is not important amino acid for pregnancy, but it is an important amino acid for the health of pregnant animals.

Oils in the Vinegar-Cakes. (pp. 833～835): By Tetutaro Tadokoro and Hiroyuki Nakayama. (Faculty of Agriculture, Hokkaido Imperial University, Sapporo, Japan; Received May 5, 1936.)

On the Inorganic Colloids in Soils (I). (pp. 836～843): S. Osugi and S. Morita. (Agricaltural Chemical Laboratory, Kyoto Imperial University; Received May 1, 1936.)

On the chemical composition of soil inorganic colloids, an extensive study has already been done by Robinson and Holmes. Bradfield, Mattson, Novàk & Smolik, Gedroiz, Iwanow, and the others have also reported some works. In Japan, Kawamura has studied the colloids separated from the alluvial and diluvial soils of middle and western districts of Japan, and Kawashima, the colloidal clay in Kyusyu district. From these works, it can be said that the caemical composition of soil inorganic colloids shows a wide variation generally but that in the restricted area, the variation is small.

The clay samples used in our present investigation were collected from various parts of Japan, extending from South Saghalien to Formosa. Colloid was separated from these thirty two clay samples by Sharples' super-centrifuge without using any peptiser, being taken 0.1 μ as the average diameter of colloid particles. Complete chemical analysis was carried out by micro-chemical method devised by Shioiri, and with the colloids from the alluvial clay samples, several main constituents soluble in HCl and Na_2CO_3 solution and free iron sequi-oxide after E. Truog were also microchemically determined.

Maximum, minimum, and mean values of the results of complete analysis of all samples are shown as follows :–

SiO_2	54.17~24.62%,	37.65%	Al_2O_3	30.44~13.95%,	21.97%
Fe_2O_3	14.92~ 5.20%,	9.00%	TiO_2	1.21~ 0.04%,	0.50%
P_2O_5	1.20%~trace,	0.32%	MnO	2.01~ 0.13%,	0.68%
CaO	5.26~ 0.38%,	1.08%	MgO	3.33~ 0.12%,	1.24%
K_2O	2.96~ 0.14%,	0.75%	Na_2O	3.93%~trace,	0.64%

The molecular ratios between silica and alumina and etc. were calculated as follows :–

	Maximum	Minimum	Averege
$\frac{SiO_2}{Al_2O_3+Fe_2O_3}$	5.69	1.17	2.41
$\frac{SiO_2}{Al_2O_3}$	5.93	1.35	3.01
$\frac{Base}{Al_2O_3}$	1.253	0.039	0.319

Calculation was made on the correlation coefficient between the various constituents and also between the constituent and some meteorological factor, and it was found that these correlations are not distinct excepting the colloids from the alluvial clays as shown in the table.

		Colloids of all samples.	Colloids of alluvial Clays:
Correlation coefficient between	$\frac{SiO_2}{Al_2O_3}$ & $\frac{Base}{Al_2O_3}$	+0.430±0.097	+0.911±0.033
	$\frac{SiO_2}{Al_2O_3}$ & mean annual temprerature	−0.397±0.100	−0.567±0.132
	$\frac{SiO_2}{Al_2O_3}$ & mean annual precipitation	+0.096±0.118	−0.559±0.134
	$\frac{Base}{Al_2O_3}$ & mean annual temperature	−0.249±0.112	−0.511±0.144
	$\frac{Base}{Al_2O_3}$ & mean annual precipitation	−0.037±0.118	−0.285±0.179

A little positive correlation was also obtained between the constituent and N-S quotient with the alluvial colloids. (Correlation cofficient between

$\frac{SiO_2}{Al_2O_3}$ & N-S quotient +0.368±0.168, $\frac{Base}{Al_2O_3}$ & N-S quotient +0.457±0.154).

On the Optical Properties of the Fermentation Lactic Acids. Part IV.

—A new enzyme Racemiase which reveals racemisation of lactic acids. (pp. 844~852): By Hideo KATAGIRI and Kakuo KITAHARA. (Agr. Chemical Laboratory, Kyoto Imperial University; Received May 6, 1936.)

In order to ascertain whether the racemisation of lactic acids caused by Lactobacilli would be observed even on their enzymatic conditions, experiments were further carried out, in the present paper, with resting state of the various Lacobacilli in the medium containing toluene and calcium lactates.

With lactobacillus plantarum sp. (*dl*-former), the velocity of racemisation was observed to be rapid and quite identical with both forms of lactic acids, therefor the *dl*-former was found to reveal direct racemisation of lactic acids.

The direct racemisation of lactic acids was again found with the other *dl*-former; Lactobacillus pentoaceticus.

Any racemisation of optically active lactic acids or any selective decomposition of racemic lactic acid was never observed with Latobacillus Sake (*d*-former) and with Leuconostoc mesenteroides var. Sake (*l*-former) even in their resting state.

Thus it was found more rigorous proof in establishing the hypothesis proposed in the previous papers, that the reason why inactive lactic acid would de obtained in natural fermentations would be due, in a great measure, to the special nature of *dl*-formers in revealing racemisation of active acids, since the active centre of the racemisation of *dl*-formers was pointed out to be a new enzyme racemiase.

On the activity of racemiase, any remarkable effect of toluene was not observed, however, racemiase was found to be rather sensitive to higher temperatures; the action of racemiase was not detected at 50° and an irreversible inactivation of the enzyme was taken place at 80°.

Technische Anleitung zur Herstellung Mannit aus Sucrose. (II).

—Anwendung des Nebenproduktes als Klebemittel einer zerriebenen Kohle. (s. 851~852): Kenji MIYAJI. (Gifu Agricultural College; Received May 14, 1936.)

Über die verschiedene Eigenschaften von Aspergillusarten. (II). (S. 853~870):

Von Y. TAKEDA und O. TAKEUCHI. (The Department of Industry, Government Reserch Institute, Taiwan, Japan; Zugeg. am 13, Apr. 1936.)

Unter Benutzung der Pfeffer'schcn künstlichen Nährbodens, dem 0.5% Oryzanin pulv. zugesetzt wurden (bei der Verwendung von Agar-Agar wurden 2% Agar-Agar zugesetzt), wurden an 89 bereits bekannten Stämmen des Aspergillus die optimale Temperatur des Wachstums, optimale Temperature der Konidienbildung, Dauer der Konidienbildung, Farbe der Konidienrasen, Farbe der Peritheziumrasen, optimale Wasserstoffionenkonzentration des Wachstums, Abtötungstemperatur, Vergärbarkeit oder Nichtvergärbarkeit von allen Arten Kohlenhydraten etc., studiert und sie nach ihrem Verhalten klassifiziert. Gegenwärtig ist die Untersuchung über andere Eigenschaften noch im Gange, daher wird eine allgemeine Klassifizierung erst nach dem Abschluss dieser Untersuchung geschehen.

ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

Studies on Canavanin. (VII).—The Synthesis of Canalin. (pp. 871～876): By Matsunosuke KITAGAWA. (Biochemical Laboratory, Department of Agriculture, Kyushu Imperial University, Fukuoka; Received June 10, 1936.)

It was shown by the synthesis of α-*o*-hydroxylamino propionic acid, $NH_2 \cdot O \cdot CH(CH_3) \cdot COOH$, that *o*-hydroxylamino fatty acid could be obtained by the condensation of benzhydroxamic acid with halogen fatty acid ester in the presence of alcoholic potash.

Author obtained α-amino γ-*o*-hydroxylamino butyric acid from the condensation of benzhydroxamic acid with α-benzoylamino γ-iodo butyric acid ester by the above method.

α-amino γ-hydroxylamino butyric acid thus obtained was identified with canalin produced from canavanin by the liver ferment, canavanase. Canalin, therefore, was confirmed to have the formula $NH_2 \cdot O \cdot CH_2 \cdot CH_2 \cdot CH(NH)_2 \cdot COOH$ as was formerly assumed, and accordingly canavanin, which has ben synthesized from canalin by the guanidation in tne previous studies, was also confirmed to have the formula $NH_2 \cdot C(NH) \cdot NH \cdot O \cdot CH_2 \cdot CH_2 \cdot CH(NH_2) \cdot COOH$.

Studies on the Assimilation of Nitrites by Fungi. (Part III.) (pp. 877～884): By Yinchang WANG. (Agricultural Chemical Laboratory, Tokyo. Imp. Univ., Received May 2, 1936.)

In the present work, the author has investigated the influences of the previous cultures and of the kinds of carbon sources upon the assimilation of nitrites by fungi.

The results may be summarized as follows,

(1) Upon the assimilation of nitrites, no influence of the previous cultures (Koji-agar; wort-agar; Czapek-agar etc.) was recognized.

(2) The nutritive value of nitrites to certain species of fungi varies according to the kind of carbohydrates contained in the medium, as seen in the following table.

++++	A luxuriant growth.
+++	A moderate growth.
++	A poor growth.
(+)	A very feeble growth.

	Saccharose	Mannit	Glucose	Fructose	Glycerine
Asp. aureus	——	(+)	++++	++++	+
Asp. awamori	+++	(+)	++++	++++	++
Asp. batatae	——	(+)	++++	++++	++
Asp. carbonarius	+++	(+)	+++	++	(+)
Asp. ficuum	+++	(+)	+++	++++	++
Asp. fuscus	+++	(+)	+++	+++	+
Asp. japonicus	——	(+)	++++	++++	+
Asp. luchuensis	++++	+	++++	++++	++++
Asp. niger	+++	++	++++	++++	++++
Asp. niger var. S.	+++	(+)	+++	++++	++
Asp. niger var. L.	+++	(+)	+++	++++	+++
Asp. pulverulentus	+++	(+)	+++	++++	+

On the Varieties of Aspergillus Oryzae employed in Shoyu-Manufacture. Part I.—Morphological Differences. (pp. 885~897): By Masajiro KIBI (Microbiological Laboratory of Yamasa Shōyu Co. Ltd., Chōshi, Chiba-ken; Received, June 25. 1936).

The writer isolated fifty-four strains of moulds from many samples of "Kōji", starter of the manufacturing of Shōyu (Japanese soy sauce).

Morphological and physiological investigations show that these moulds belong to twenty-eight varieties of Aspergillus Oryzae.

The following is the morphological characters of numbering 28 varieties.

General characters:— Colonies green; Stalks colorless and not septate; Outer wall of stalks pitted or rough (except No. 6); Vesicles globose or subglobose; Sterigmata in 1 series; Conidia globose; Conidial wall smooth at first (except No. 6).

Varieties	Length of stalk (mm)	Diameter of Conidia (μ)	Synopsis
No. 1	0.2	3.5~4.5	Colonies velvety; Conidia develop quickly.
No. 2	0.2	3~3.5	Heads forming long columnar masses consisting of parallel adherent chains of conidia.
No. 3	0.3	3.5~4	
No. 4	0.4	3.5~4	
No. 5	0.4	3.5~4	
No. 6	0.6	5.5~6.5	Conida roughened; stalks smooth

No. 7	0.6	4~5	Heads forming columnar masses consisting of parallel adherent chains of conidia
No. 8	0.7	4~5	
No. 9	0.9	6~7	Heads nearly spherical
No. 10	1	4~5	Heads forming resemblingly to No. 7, still shorter
No. 11	1.1	4~5	
No. 12	0.9~1.3	5.5~6.5	Conidial chains long; heads large-spherical
No. 13	1.1	7~9	Colonies slightly brownish green
No. 14	1.3	5.5~6.5	Heads hemispherical
No. 15	1.3	5.5~6.5	Heads forming resemblingly to No. 7, still shorter
No. 16	1.4	5.5~6.5	Heads large-spherical
No. 17	1.6	6~7	Sclerotia found
No. 18	1.6	6~7	Heads nearly spherical
No. 19	1.6	4~5	Heads spherical
No. 20	1.7	6~7	Growing at low temperature, colonies light-colored
No. 21	1.7	6~7	No conidia often develop
No. 22	1.7	7~9	Colonies grayish green
No. 23	1.8	6~7.5	Stalks big
No. 24	1.8	6~7	Stalks slender
No. 25	1.8	6~7	Close resemblance to No. 24
No. 26	1.6~2	6~7.5	Stalks thin; conidia develop slowly
No. 27	2	6~7	Heads nearly spherical
No. 28	2.2	8~10	All organs gigantic

Separation of Cyanuric acid from the Soil of "Kagamigahara"

(pp. 898~899): By Chikafumi ICHIKAWA. (The Soma Agricultural and Sericultural School; Received June 2, 1936)

The presence of Cyanuric acid in soil has been assertained by several students, of whom Messrs. Kasugai and Ozaki have reported very instructive results of their joint studies on the same fact.

Following their method, the present author has cleared up the presence of Cyanuric acid in the soil of Kagami-ga-hara, near the city of Gifu.

A Chemical Interpretation of the Difference of Colours on Leaf and Flower.

(pp. 900~908): By Chikafumi ICHIKAWA. (The Soma Agricultural and Sericultural School; Received May 26, 1936.)

Application of Mycellium of Aspergillus Oryzae exposed to the Ultra-violett Ray on Poultry (I).—Effect on the Growth of Chicken.

(pp. 909~915): By Ryohei TAKATA and Kisaburo YOKOYAMA. (The Osaka Imperial University; Received May 18, 1936.)

On the Inorganic Constituents of Green-Manure Crops. II.

(pp. 916~930): By Kametaro KONISHI and Toshihisa TSUGE. (Agr. and Forest Chemical Laboratory, Kyoto Imperial University; Received July 11, 1936.)

(1) Biological studies on the respiration of *Rh. meliloti* apart from the host plant in nutritive media and in buffer solutions with addition of the titanium salts in different quantities.

(2) Vegetation experiments on the effects of titanium salts upon the growth of not inoculated alfalfa without nodules in water cultures containing Crone's salts plus natrium nitrate.

(3) Estimation of mineral consitituents of Mycorrhiza and Crown gall from certain non leguminous plants in comprison with alfalfa nodules.

Über die Gärungsmikroorganismen in Awamori-Bereitung (I.)—Aspergillus-Arten.

(S. 931~974): Von R. NAKAZAWA, M. SIMO und H. WATANABE. (The Department of Industry, Government Reserch Institute, Taiwan, Japan; Zugeg. am 4. 6. 1936.)

Man hatte bisher geglaubt, dass die wichtigeren Schimmelpilze, die sich an der Awamori-Gärung beteiligen, nur die beiden folgenden sind, nämlich Aspergillus Awamori und Aspergillus aureus; aber in dieser Untersuchung wurden 14 Arten Aspergillus isoliert und im folgenden seien die wichtigeren 12 Arten Aspergillus angeben:

Aspergillus Awamori var. minimus nov. var.
Aspergillus Awamori var. piceus. nov. var.
Aspergillus Awamori Nakazawa.
Aspergillus Awamori var. ferrugineus nov. var.
Aspergillus aureus var. minor nov. var.
Aspergillus Awamori var. fuscus nov. var.
Aspergillus aureus var. murinus nov. var.
Aspergillus aurews var. acidus nov. var.
Aspergillus Awamori var. fumeus nov. var.
Aspergillus aureus var. pallidus nov. var.
Aspergillus aureus var. brevius nov. var.
Aspergillus miyakonesis nov. sp.